Reference: *Biol. Bull.*, **143**: 162–174. (August, 1972)

A HIERARCHY OF HISTO-INCOMPATIBILITY IN *HYDRACTINIA ECHINATA* [1]

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There is a striking consistency in the biochemistry and ultrastructural morphology of all living cells. These cells do, however, recognize differences among themselves and react accordingly. Cellular recognition mechanisms are operative in dissociated embryonic cells derived from different organisms (chick and mouse), so that cells of like function remain together in chimeric aggregates, while those derived from different organs segregate (from each other) (Moscona, 1957). Dissociated sponge cells segregate according to species (Humphreys, 1963), and cells from different tissues of the same organism segregate from each other within the initial reaggregate mass (Steinberg, 1962a, 1962b, 1963). Mechanistic explanations of cellular segregation focus on differences in cellular adhesiveness (Townes and Holtfreter, 1955) due to stereospecificity of binding sites; species specific extracellular binding molecules (Humphreys, 1963); or variations in thermodynamic energies of adhesion (Steinberg, 1962a); or specific recognition sites on cell membranes for specific histocompatibility antigens (Burnet, 1970).

Recognition and interaction exist between unlike cells as well. Endocrine secretions affect specific target organs. All inductive processes involve molecular mediators. Normal development is a well integrated temporal series of inductive interactions in which one tissue chemically initiates change in a second tissue. All differentiation and morphogenesis is the result of delicately balanced intra- and and intercellular stimulation and feedback control systems. Occasionally there is a breakdown in the system, resulting in hyperplastic or neoplastic growth.

The study of developmental deviations in simple organisms may reveal the mechanisms of similar imbalances in more complex species. The Coelenterata offer a simple system in which to approach these developmental mechanisms.

The main focus of this study is the "overgrowth" phenomenon, a hyperplastic development of stolons resulting from a histo-incompatibility among genetically different strains of *Hydractinia echinata* isolated from nature. Some attention will also be given to some aspects of normal development which have been misinterpreted in the literature.

Materials and Methods

Hydractinia echinata is an encrusting, colonial marine hydroid usually found on gastropod shells that have been appropriated by the hermit crab *Pagurus*. All

[1] This investigation was supported in part by National Institutes of Health Grant GM 11555 and a grant from the Indiana University Foundation.

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strains used in this study were isolated from shells collected in the Woods Hole, Massachusetts area.

A hierarchy of overgrowth potential was established twice, using two sets of ten strains each. The first group of animals (Group 1; 1–5 male, 6–10 female) was started from animals scraped from the surface of shells collected in August at the time when the colonies were in full sexuality. The second group (Group 11; 1–5 male, 6–100 female) was collected in December when the colonies showed juvenile or regressive sexual development. Clones derived from the mating of these colonies were designated by the number of both parents (*i.e.*, colony 8 mated to colony 4 produced 8–4 offspring). In the F_1 generation, the overgrowers were designated 8–4(0) and their overgrown siblings were labelled 8–4(X).

Stock strains were started by scraping a piece of mat from a colony growing on a hermit crab shell. One or two feeding hydranths were then teased from this mat and their proximal, cut ends held in close contact with a glass slide by a single loop of thread tied around the slide. Within 24 hours in the faster growing strains, stolons grew out of the cut ends, adhered to the glass and thereby held the feeding hydranths to the slide. The thread was then removed. Three to five colonies were started under each thread. Stolons grew out from the transplanted hydranths so that they eventually met stolons of the other transplants. If the colonies were of the same stock, they fused, forming a continuous colony across the width of the slide. If the transplants were of different stocks, hyperplastic stolons were produced by one or both of the colonies.

The first group of experimental animals was raised on glass slides, held vertically in glass staining racks in standard ($2\frac{1}{2}'' \times 3'' \times 3''$) staining dishes. The dishes were placed on a slow, gentle horizontal shaker. The second group of animals was grown on slides in staining racks that were placed in a plexiglass tank $3'' \times 15'' \times 3''$. Water was circulated by a vibropump and passed through glass wool and charcoal filters.

The hydroids were kept in natural sea water that was pasteurized by heating to 80° C on two consecutive days and aerated for 30 minutes before use. The water was changed every 7 days. Temperatures ranged between 19° and 22° C during the course of the study. The cultures were fed freshly hatched brine shrimp (*Artemia salina*) once a day.

Precautions were taken to keep the cultures as "clean" as possible, but due to their living food and their initial isolation directly from nature, the cultures were occasionally infected by bacteria, algae and ciliated protozoa. The contaminants were kept in check by periodic light swabbing of the slides and colonies with cotton wound on a thin glass rod. Heavy infestations of ciliates were treated by placing the cultures in a dilute (100 units/ml) solution of Mycostatin for 20 minutes, which cleared the slide of surface ciliates without apparent harm to the hydroids. Heavily infected areas of the colonial mat were cut away and the excised tissue was quickly replaced by healthy new tissue.

In order to test strain compatibility, two strains were placed on a slide and allowed to grow until the stolons contacted each other. All possible binary combinations (45) of the ten strains in Group I and Group II were tested. Compatibility was also tested on gastropod shells to insure that the overgrowth phenomenon obtained in nature as well as in the laboratory. Hermit crabs were removed from

their shells and kept in isolation. Hydranths of the strains to be tested were tied in place. When the colonies appeared fastened to the surface by stolonic growth, the threads were cut and the crabs were allowed back into their shells. Each crab was kept in its individual finger bowl to avoid abrasive contact with other crabs.

Vital staining was accomplished by feeding stained *Artemia* nauplii to the colony to be dyed. One drop of either 0.1% aqueous Nile Blue Sulfate or Neutral Red was added to 30 ml of sea water. Freshly hatched *Artemia* was left in this staining medium for 24 hours. They were then picked in a hand-held micro-pipette and presented within the tentacle range of the colony to be stained: red to one colony and blue to the other colony of a binary combination. Saturation feeding once a day for 6–10 days produced enough color in the growing stolons to determine the origin of solons in an overgrowth tangle and to detect any exchange of material between the colonies. Reciprocal staining procedures were initiated in several incompatible pairs to negate the possibility of chemical involvement of the dye in hyperplastic stolon development. When normal feeding was resumed, the vital dyes faded in about two weeks without apparent harm to either colony.

Controlled breeding was accomplished by isolating sexually mature female colonies at least two days before mating. A slide containing a male colony was introduced into the staining dish, facing the female colony at a distance of 1–2 cm. Eggs were observed on the bottom of the dish the next morning and elongated planula larvae 12–18 hours later. If larvae were allowed to remain scattered on the bottom of the breeding dish, metamorphosis into small four-tentacled, feeding hydranths was observed in 10–40% of the larva in 7–21 days. Larvae were also picked up in a micro-pipette and introduced onto the clean shell of a hermit crab, resulting in a higher rate of metamorphosis (30–60%). When a new colony was well established, either on glass or shell, one or more feeding hydranths of normal size were transferred to a glass slide, as described earlier, and a stock colony established. The conditions responsible for the induction of sexuality and metamorphosis are highly unpredictable at this time. Most breeding was done at Woods Hole in July and August, but crosses have been made in Indiana in December, and Crowell (1950) and Hauenschild (1954) regularly raised sexually mature colonies in mid-winter at Tübingen, Germany.

Results

Normal growth

Within twenty-four hours after placing a newly isolated hydranth in contact with a slide, stolons grew out along the glass surface, firmly attaching the hydranth to the substratum. Stolons grew in all directions, branching and anastomosing freely within the two dimensions of the slide. They displayed no gravitational tropisms and always maintained contact with the substratum. Other hydranths sprang up along the stolons, increasing the feeding and growth potential of the new colony. The area between the branching and anastomosing stolons was subsequently filled with tissue, histologically described (Berrill, 1953; Bunting, 1894) as an ectodermal mat penetrated by interconnected, endodermally lined gastrovascular channels.

The rate and pattern of stolon growth and mat formation varied among the different strains being raised under identical culture conditions. This fact was also noted by Schijfsma (1939). The same growth patterns appeared in all colonies derived from the same original colony isolated from nature.

Alterations in the culture conditions brought about changes in these developmental patterns. Lack of water movement, reduced aeration and infrequent water changes led to a retardation of stolonic outgrowth, but not to a concomitant reduction in the rate of hydranth production. The result was a smaller colony with a greater density of nutritive zooids. Return of the colony to standard culture conditions produced a renewed stolonic outgrowth similar to that seen in control colonies of the same strain. Cleaning the slide surface and cutting away infected perisarc restored normal fusibility.

Growth on the surface of a gastropod shell was slower than on glass, and mat formation followed more closely behind stolon growth. In shells occupied by a crab, spines were produced after three or four weeks, but on empty shells no spines were produced, although the colony grew well and even reached sexual maturity in some cases.

Overgrowth (hyperplastic stolons)

In order to test compatibility, colonies of two strains were started on a slide, as described earlier. Each developed normally, sending branching, anastomosing stolons in all directions, always maintaining contact with the glass surface, until stolons of one colony made contact with stolons of the other colony. At this point, one of the colonies started to produce abnormal stolons; they rose up off the slide, losing contact with the substratum, and formed hyperplastic, tangled masses. They did not immediately fuse with other stolons of the same colony as they normally would have done when growing flat on the glass surface. During all the tangling, they maintained the general direction of growth toward, and over the other colony. The hyperplastic stolons grew over the stolons, mat and feeding hydranths of the other colony, cutting off its contact with the food supply and eventually causing its death.

Closer observation of the overgrowth phenomenon indicated that physical contact of incompatible strains was essential to the induction of hyperplastic growth, and only those stolons in contact with the overgrown colony were affected. Although material was seen to circulate throughout a colony via the gastrovascular system, hyperplastic stolons were not observed in other areas of the overgrowing colony. Attempts were made to induce the production of stolonic overgrowth throughout a colony by immersing it in a crude brei made from another colony known to induce overgrowth by the contact method, but no positive results were obtained.

Although no abnormal growth patterns were observed in other areas of a colony involved in overgrowth production in the contact stolons, it was noted that normal growth was quantitatively reduced, while the rate of growth of stolons actively involved in overgrowth was increased. The colony as a total unit appeared to be concentrating its corporate nutritive resources in the production of the stolonic tangle at the expense of normal growth in other areas. It was also

observed that colonies actively involved in the overgrowth process showed delayed sexual maturation, as compared to control colonies of the same strain.

A tangled mass of stolons could reach a height of 5 mm above the surface of a slide and extend 25 mm across an overgrown colony. When these hyperplastic stolons completely covered the underlying colony and reached the glass surface on the far side, they immediately returned to their normal, two-dimensional, anastomosing growth pattern, regardless of hyperplastic growth still in progress in lateral areas not yet completely covered.

The overgrown colony did not die immediately. First, the feeding hydranths were reabsorbed in the area initially covered by the stolonic tangle. As the overgrowth progressed, more and more nutritive zooids were reabsorbed until all were gone, leaving the overgrown colony with no means of obtaining food. There were, however, large food reserves within the mat, and if the overgrowth was stripped away before too much of these reserves had been utilized, the overgrown colony could again produce feeding hydranths and resume normal growth with no obvious ill effects. If the tangle remained, the tissue of the overgrown mat slowly utilized its food reserves and died within 3–6 weeks. During this time, the stolonic tangle above remained static, while normal growth and hydranth production continued on either side of the tangled mass. Anastomosis of the upper surface stolons of the tangle was observed at about the same time we assume death occurred in the underlying tissue. Observation of cross sections of the mass, using the dissecting microscope, revealed a spongy center of empty perisarc that formerly contained stolons of the overgrowth. The surface was a continuous mat of living ectoderm containing numerous channels lined by endoderm. These channels became clearly defined when vital stain was applied via the food source.

Spontaneously, over the entire irregular surface of the mass, feeding hydranths appeared, with the same density and morphology as those seen in areas of normal growth. If the colony had shown retarded sexual development, gonozooids appeared, evenly distributed on the flat, normal mat and on the surface above the tangle.

No transfer of colored material could be detected between incompatible colonies that had been vitally dyed in contrasting colors, even as the overgrown colony diminished in volume, no absorption of colored material was noted in the overgrowing colony.

Colonies derived from the same source retain their compatibility even after long periods of separation. Fusion was observed between colonies derived from two older colonies of strain #8, Group II, which had overgrown strain #2 and strain #7 respectively, and had been isolated as individual colonies for ten months. Their temporal separation and physiological activity in the overgrowth process had not interfered with or altered their compatibility (fusibility). In similar tests, hydranths from the top of a tangled mass were explanted to a slide between explants of the two strains whose interaction had given rise to the tangled mass of stolons. In all nine cases, the tangle explant colony fused with the colony that had produced the overgrowth and it, in turn, produced hyperplastic stolons when contact was made with the overgrown strain, again indicating no alteration in tissue compability as a result of participation in the overgrowth process.

In rare instances, both colonies produced abnormal stolons upon contact, but one eventually outproduced and overgrew the other. There were situations in

which related, but not genetically identical colonies, such as parent and offspring, or two strains with one common parent, contacted each other; each produced a limited number of abnormal stolons, which were quickly replaced by an abnormal, thickened area of mat tissue on both sides of the line of contact. Vital staining showed that there was no fusion or transfer of material between the two colonies, but neither was there any hint of overgrowth by either one or the other.

Hierarchy

In selecting ten colonies at random from nature and setting up all (45) possible binary combinations, it was observed that if colony A overgrew colony B, and if colony B overgrew C, it could be predicted that colony A would overgrow colony C. There was a definite, predictable hierarchy of overgrowth, the strongest

TABLE I

Results of binary combination—Group I

Strain	Sex	Overgrows	Is overgrown by	Rank in hierarchy
2	♂	1 3 4 5 6 8 9 10	[7]	1
5	♂	3 4 6 7 8 9 10	2 (1)	2
3	♂	1 4 6 7 8 9 10	2 5	3
8	♀	1 4 6 7 9 10	2 3 5	4
7	♀	1 [2] 4 6 9 10	3 5 8	5
1	♂	4 (5) 6 9 10	2 3 7 8	6
9	♀	4 6 10	1 2 3 5 7 8	7
4	♂	6 10	1 2 3 5 7 8 9	8
10	♀	6	1 2 3 4 5 7 8 9	9
6	♀		1 2 3 4 5 7 8 9 10	10

□ + ○ are not in expected positions.

overgrower being listed as #1 in the hierarchy. The strain that was overgrown by all the others was designated as tenth in the hierarchy. The other strains all fell in order, depending on their relative frequency as an overgrower.

In the first set of data presented in Table I, there are two discrepancies in the hierarchy. Strain #7 was scored as overgrowing #2, and #1 was scored as overgrowing #5, which is contrary to expectations based on data derived from the other 43 binary combinations in this group. Neither of these results could be checked due to an accidental loss of all Group I strains. There was one apparent discrepancy in the predicted results of Group II. In the first trial, #7 appeared to overgrow #8, but subsequent tests of the pair, both on slides and crab shells, accompanied by vital staining, proved #8 to be the overgrower as would be predicted from other tests, leading to the possibility that there may have been a labeling reversal in the initial trial.

Although two strains proved incompatible upon stolonic contact, this did not affect sexual interaction. It was, therefore, possible to produce second generation strains. There were five matings in Group I and three in Group II.

The larvae were allowed to metamorphose on the bottom of the breeding dish. As growth continued it was observed that stolonic compatibility and incompatibility existed between siblings and that either fusion or overgrowth occurred at the junction of any two colonies. By transferring colonies to slides and setting up from nine to twenty-four possible combinations per mating, it was determined that only two classes of offspring existed: the overgrowers (*i.e.*, 7–4(0)) and the overgrown (7–4(x)). All those that overgrew their siblings were compatible, and all those that were being overgrown were fusible with each other. Because of the large numbers of larvae and limited space, no accurate determination of the percentage of offspring in each class was made.

TABLE II

Results of binary combinations—Group II

Strain	Sex	Overgrows	Is overgrown by	Rank in hierarchys
10	♀	1 2 3 4 5 6 7 8 9	—	1
8	♀	1 2 3 4 5 6 7 9	10	2
9	♀	1 2 3 4 5 6 7	8 10	3
4	♂	1 2 3 5 6 7	8 9 10	4
1	♂	2 3 5 6 7	4 8 9 10	5
7	♀	2 3 5 6	1 4 8 9 10	6
6	♀	2 3 5	1 4 7 8 9 10	7
5	♂	2 3	1 4 6 7 8 9 10	8
3	♂	2	1 4 5 6 7 8 9 10	9
2	♂	—	1 3 4 5 6 7 8 9 10	10

DISCUSSION

The initial work on *Hydractinia* was basically descriptive of normal development (Bunting, 1894; Teissier, 1929; Teissier and Teissier, 1927; Schijfsma, 1935, 1939; Berrill, 1953). Teissier (1929) and Schijfsma (1939) noted that there was fusion into a single colony when stolons derived from different planula larvae made contact, but an anomaly was noted by Schijfsma in 1939 (page 101): "It looks as if the growing borders of two colonies, in striking together and checking each others progress, are stimulated to very active growth and ramifications; resulting in the formation of a dense fringe of intertwined stolons."

Schijfsma vaguely speculated about a "timing factor" but noted that this "fringe" did not appear when a colony met itself on the other side of a shell. This indicated that the "fringe" was not a normal marginal phenomenon. Toth (1967) enlarged upon this suggestion, calling it "temporal specificity" (page 131), and claiming that compatibility, even of clonal colonies was variable with time. He stated that all colonies were compatible early in life, but became increasingly selective; eventually colonies of the same strain could not fuse. In this study, however, incompatibility was demonstrated between newly metamorphosed (2–3 day) colonies by the production of hyperplastic stolons which overgrew sibling colonies; while continued compatibility was demonstrated by the fusion of explant colonies derived from two colonies of the same strain that had been established as individual colonies ten months earlier. Both colonies had undergone the physio-

logical stresses of overgrowth production and still retained their compatibility. Colonies derived from hydranths on the surface of the tangle fused with clonal colonies of the strain that had originally produced the tangle, indicating that even stolons that initially appeared unable to fuse during the overgrowth process retained their histo-compatibility when they returned to normal colonial metabolism.

Another point of variance in Toth's paper (1967) was his report that colonies on glass slides usually reached a maximum diameter of 5–10 mm before the "endogenous limit of closed periderm is attained" (page 131). He does, however, mention later that no such limit is seen in nature. No such limits were seen in the present study. A "dirty" slide whose surface is covered by a layer of bacteria, algae and/or protozoa will inhibit or halt free stolon growth. The vulnerable stolon, with its high surface-to-cell-mass ratio, is poisoned or damaged by these other organisms faster than it can regenerate new tissue, and eventually new stolon growth stops. The mat may slowly expand for a while longer under these conditions, but this too eventually ceases. Toth's (1967) description of a "limiting periderm" and reduced stolon growth can be explained as an artifact of substandard culture methods. His report of incompatibility (lack of fusion) between colonies of the same strain may be due to a build-up of necrotic tissue or bacteria-encrusted perisarc at the contiguous margins of the two colonies, preventing perisarc dissolution by ectodermal enzymes or preventing cell-to-cell contact and tissue fusion.

Toth (1966, 1967) reported free stolon growth in 10% of his strains. Both Hauenschild and Kanellis (1953) and Toth (1967) suggest this may be due to poor nutrition. The current study of well fed and well aerated stocks produced an open stolon pattern in 80% of the strains tested, with a varying stolon/mat ratio characteristic of each strain and reliably reproduced by all colonies derived from that strain. Reduced oxygen supplies in the medium retarded stolon growth. The mat continued to grow as a slowly expanding circle from the point of implantation. A return to more advantageous culture conditions brought a renewed outgrowth of freely anastomosing stolons.

Crowell (1950), Hauenschild (1954), and Toth (1967) discuss lack of fusion between strains and regard this as incompatibility, but none of these investigators records or discusses the induction of hyperplastic stolons. Müller (1964), however, does report the formation of stolonic "knots" to which both colonies contributed stolons.

The role that particular strain plays in relation to any other strain, either as overgrower or overgrown colony, is not a chance occurrence. Among the strains tested, a very definite hierarchy emerged in both sets of experiments.

There appeared to be a correlation between growth potentials, as related to colony morphology, and the position of a strain in the hierarchy. A fast-growing, highly stolonic strain is likely to rank higher on the scale of overgrowth potential than a slower-growing, short-stolon, large-mat former; the correlation is not absolute, however, and rank by growth rate becomes difficult to determine among strains of similar developmental morphology.

Vital staining experiments showed that in some cases both colonies initially produced abnormal stolons. Müller (1964) reported the participation of both colonies in the formation of a stolonic "knot." In almost all cases, one strain was superior in hyperplastic stolon production and the other began a regression that

ended in death. Müller suggests that this regression is caused by a toxin produced by the overgrowing colony. Stripping away the overgrowth leads to rapid (2–4 days) and full recovery, suggesting that the regression is the result of mechanical stresses applied by the hyperplastic stolons. When 80% of the colony being overgrown is covered, the other 20% spontaneously withdraws the feeding hydranths in the uncovered area. It is suggested that this is the result of a general physiological regression of the entire colony caused by an unfavorable balance between metabolic requirements and nutritional acquisition rather than a reaction to a specific toxin.

The term "hierarchy" suggests the work of Steinberg (1963) and his hierarchy of embryonic tissue associations and segregations in tissue culture. His results indicated a predictable position in a cellular reaggregate. Pre-cartilage had the highest probability for interior position, liver the highest probability for the outside, with heart-cells variable, based on the particular binary combination. Steinberg repeated these experiments with several embryonic tissues establishing a hierarchy of potential position at the center of the mass. The explanation offered by Steinberg involved an "energy of adhesion" between cells, so that if two cells of type A displayed a significantly higher attraction for each other than did those of type B or an A cell for a B cell type, the A type cells would tend to aggregate together with as much mutual surface contact as possible, thereby excluding cells of the B type from their midst and forcing them toward the periphery of the reaggregate cell mass.

"Energy of adhesion" is a cell surface phenomenon in which cells seek the "lowest energy state" or most stable adhesive condition possible. The adhesive mechanism could, in principle, be a quantitative one based on the number of adhesive sites available or a qualitative one based on the specificity of the various sites.

Applying these hypotheses to the hierarchy in *Hydractinia*, two possible mechanisms may be proposed, both involving a surface-bound molecule produced by the ectoderm. The first hypothesis involves a quantitative variation in this substance; the second suggests a qualitative difference. When stolons of the same strain meet, the quantity and/or quality of the molecule is identical and fusion results. If, on the other hand, there is a significant difference in either quantity or quality of the substance, the stolons recognize this difference and react by the production of hyperplastic stolons by one or both colonies.

Looking first at the qualitative hypothesis, we can postulate a mechanism similar to serotypes found in *Paramecium*. (Sonneborn, 1948). Incompatible strains would produce strain-specific proteins which could induce hyperplasia in other strains. The intensity of the reaction could be due to the degree of difference in the surface molecule. It was noted that the intensity of incompatibility, as indicated by the speed and quantity of induced hyperplastic growth, varied considerably among the strains tested. Certain slow-growing strains, such as 4, 6 and 10, Group I and strains 2 and 3, Group II, induced a much weaker reaction from the #1 strain in their respective hierarchies than did other overgrown strains higher up in rank.

The maximum degree of difference that will trigger the reaction is limited, however, as evidenced by the fact that contact with other hydroids (*i.e.*, *Campanularia*, *Bougainvillia* and *Podocoryne*) failed to elicit a response. This indicated that the overgrowth reaction was not a simple antigen-antibody-like response to a

foreign protein, but rather a highly specific, intra-species selective mechanism that plays a role in genetic distribution within the *Hydractinia* population.

Considering the quantitative difference hypothesis, the colony with the higher concentration of the particular molecule in any pair might be the inducer. This would explain a strain's shift from overgrower to one which is overgrown as a shift in the relative amount of this surface molecule, when compared to the quantity of this substance in the other strain of any particular combination. This would be similar to Steinberg's hypothesis of differential adhesion based on a quantitative difference in available binding sites. The hierarchy then would be a quantitative ranking of the presence (or absence) of this inducer molecule.

Preliminary experiments (Ivker, 1967) with reaggregation of dissociated, stained endoderm cells do not demonstrate histo-incompatibility between strains on the cellular level.

Braverman, M. (Allegheny General Hospital, Pittsburgh, Pa.) has photographic and histologic evidence that even in normal stolon fusion, in the related encrusting species *Podocoryne*, the advancing stolon tip produces a substance that causes an increase in the size of the epidermal cells that the tip is about to contact. An increase in epidermal cell size is also seen in the overgrowth stolons. Müller (1964) mentions a hyperplasia of epidermal cells in both the stolons and the mat in the area of contact between incompatible strains.

It is suggested that each strain produces a substance (in greater quantity at the growing tip) which has a hyperplastic effect on epidermal cells. In the contact of stolons of the same colony, or colonies of the same strain, the stolon tip is thought to produce an enzyme which dissolves the perisarc in a small area and facilitates cell-to-cell contact and fusion of the gastrovascular cavities. When strains are incompatible, the surface substance on the growing stolon again induces a reaction in the epidermal cells in the area of contact. These cells produce more of their own surface substance which in turn induces hyperplastic growth in the oncoming stolons. This accelerated growth rate may prevent the accumulation of sufficient enzyme at the stolon tip, thereby preventing the fusion of actively growing hyperplastic stolons with each other. Müller (1964) proposes a similar mechanism (page 241) when he ascribes "wild" stolon growth to a stimulation of dormant developmental potential in one strain by a foreign (incompatible) strain.

Attempts to characterize the inducing substance have given rise to several hypotheses. It is either bound to the ectodermal surface or it slowly diffuses through the perisarc from the ectoderm. The failure of an incompatible strain to "condition" the medium in which it was grown, and the failure of a crude brei of incompatible colony to induce hyperplastic stolon development may be evidence of the small quantities produced by any given colony and/or the failure of the material to reach a concentration above the threshold required for hyperplastic induction.

As was noted earlier, stolonic contact between incompatible strains was essential for the induction of hyperplastic stolons. Only growing stolons were affected (*i.e.*, those laid down before contact was made remained unaffected). Nothing was carried via the gastrovascular system to induce abnormal stolon development in any area of the colony not in direct contact with the overgrown colony, leading to the hypothesis that the overgrown colony acts as an inducer. The inducing agent is either surface bound or a large molecule that cannot easily diffuse through

tissue or water in sufficient concentration to induce overgrowth in any but contiguous stolons. There must be continued production of the substances, since the stolonic mass built up, but remained as a deep tangle of stolons as long as there was living inducer colony below it. Stolons that reached the far side of the overgrown colony and made contact with clean glass, resumed their normal growth pattern and regular hydranth production. Although stolons in the tangle acted as connectives between colony mat on either side of the overgrown colony, no inductive material was transported in either direction to affect hydranth production. Hyperplastic growth was localized to that area in direct contact with the incompatible colony. The only area lacking feeding hydranths and not displaying stolon anastomosis and mat formation was the tangle mass itself and this situation was temporary. The eventual stolonic fusion, mat formation and appearance of nutritive polyps was believed to coincide with the death of the overgrown colony and the cessation of its production of inducer. It is suggested that the inducer substance promotes increased growth (*i.e.*, hyperplastic stolons that do not anastomose) yet it inhibits differentiation of specialized tissue areas like feeding or reproductive zooids. Perhaps the increased growth rate prevents the concentration of material required for hydranth formation.

Although there was no gross morphological change observed in the outlying parts of the overgrowing colony, there was, nevertheless, an effect felt throughout the colony. The hyperplastic mass resulted from an accelerated deposition of material in one area, which could only be made at the expense of growth in other areas. The stolonic tangle increased the mass of the colony, but the nutritive capacity of the colony did not undergo a concomitant increase, due to the absence of feeding hydranths in the tangle area. There was, therefore, a definite decrease in peripheral stolon growth and mat production as compared to control colonies. There was not total cessation of normal growth patterns, but a noticeable retardation as the colony concentrated its productive energy and nutritional resource in the overgrowth mass.

Although the induction of sexuality is far from understood, optimal nutrional conditions are a prerequisite to the process. Sudden starvation caused the transformation of gonozooids into nutritive polyps. The reverse was not true. Gonozoiids arise *de novo* from the mat tissue and were not derived from pre-existing nutritive structures. It is postulated that the accelerated proliferation of stolon tissue that constituted the overgrowth mass depleted the nutritional reserves necessary for gonozooid production, thereby retarding sexual differentiation.

Sex does not appear to be related to compatibility. Colonies initially determined to be 7–5(X), Group I immediately after metamorphosis, but raised on separate slides, subsequently turned out to be of opposite sex. Fusion of these colonies after initial growth had established the individuality of each colony could produce sexual chimeras in the fusion zone, as reported by Müller and Hauenschild. Müller (1964) reported having to try many binary combinations (which produced "knots") before he found two strains that were even partially compatible. In this case, he reported fusion of the endodermal gastrovascular cavities, while the mat ectoderm of the female colony formed large masses that appeared to invade the male colony. The masses eventually withdrew. The separation of apparently fused, partially compatible colonies was observed in this study, especially in times of physiological depression. In trying to localize the source of incompatibility, it

was found that empty perisarc did not elicit a reaction, indicating that the inductive substance was not an integral part of the molecular structure of the perisarc.

The variability of morphology seen between various strains, the consistency of morphology within a strain and the clear-cut orderliness of the hierarchy indicate a genetic control mechanism. Hauenschild initially proposed a single locus, six-allele system to explain the results of his histo-incompatibility studies, but ultimately abandoned the hypothesis when it proved inadequate to deal with the complexity of accumulated data.

Looking to other biological systems for a clue, the colonial tunicate *Botrylus schlosseri* presents a seemingly similar example of histo-incompatibility in which the vascular systems of incompatible strains fail to fuse, but no hyperplastic growth is observed. Karakashian and Milkman (1967), Milkman (1967) postulate a multi-allelic system, but assign no definite number of alleles.

At the present time, there is no definite evidence to support a multi-allelic versus a multi-genic hypothesis, or to rule out a complex combination of the two. With the demonstrated feasibility of controlled laboratory mating and the use of morphologically unique strains, it is hoped that some insights will soon be gained into the mechanisms of the genetic transmission of incompatibility.

The ability to produce hyperplastic stolons and a high place on the hierarchy appears to have a selective value in nature. It is hoped that morphologic markers can soon be found that can help trace the ecological distribution of the genetic factors responsible for the overgrowth phenomenon, and that the inductive mechanism can be more firmly established.

Summary

1. *Hydractinia* represents a simple system in which to study induction, cellular-recognition mechanisms, and hyperplastic growth.

2. Among various strains isolated from nature, there is a tissue incompatibility upon contact which results in the production of hyperplastic stolons (overgrowth) in one or both colonies of any binary combination of strains. The induction of hyperplasia probably involves surface-bound molecules produced by the ectoderm.

3. A hierarchy of hyperplastic potential was established in two groups of ten strains each. A correlation between colonial morphology and rank in the hierarchy was noted.

4. Consistency of morphology and intermediate forms of incompatibility between related strains (*i.e.*, parent-offspring, half-sibs) suggests genetic control of histo-incompatibility and hyperplastic growth.

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